# Lupine ELISA Kit

Enzyme Immunoassay for the quantitative determination of Lupine in food

Catalog number: ARG80804

Package: 96 wells

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## INTRODUCTION

Lupin belongs to the legume family. With about 35-45% the fraction of proteins in lupin seed is very high. Some of these proteins, predominantly those in the α-, β- and γ-Conglutin fractions are known for being allergenic, due to cross-reaction to allergens of soy and peanut. Because of its convenient nutritional properties the use of lupin flour as a food ingredient is highly increasing. Lupin is often used in dietary products, especially for milk allergic people, vegetarians and patients with celiac disease. In food production three (sweet) lupin species are commonly used: Lupinus albus, Lupinus angustifolius and Lupinus luteus.

For lupin allergic persons hidden lupin allergens in food are a critical problem. Already very low amounts of lupin can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, lupin allergic per-sons must strictly avoid the consumption of lupin containing food. Cross-contamination, most-ly in consequence of the production pro-cess, is often noticed. This explains why in many cases the existence of lupin residues in food cannot be excluded. For this reason sensitive detection systems for lupin residues in food-stuffs are required.

The Lupin ELISA represents a highly sensitive detection system for lupin and is particularly capable of the quantification of residues in sausage, bakery products, potato products, ketchup and juices. Due to high cross-reactivity the test is suitable for the detection of all commonly used lupin species.

## PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody directed against Lupine is bound on the surface of a microtiter plate. Lupine containing samples or standards are given in-to the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against Lupine proteins is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Lupine is directly proportional to the color intensity of the test sample.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody-coated microplate | 12 strips x 8-well | 4°C |
| HRP-antibody Conjugate | 15 ml (ready to use) | 4°C |
| Standards (0,2, 5, 15, 30 ppm) | 5 X 2 ml (ready to use) | 4°C |
| 10x Extraction and sample dilution buffer | 2 X 120 ml | 4°C |
| 10x Wash Buffer | 60 ml | 4°C |
| TMB substrate | 15 ml | 4°C (Protect from light) |
| STOP solution | 15 ml | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

## SAMPLE COLLECTION & STORAGE INFORMATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. Hazelnut proteins adhere very strongly to different surfaces. In certain cases they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

**The following sample preparation should be applied for solid samples:**

1. To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2. 1 g of the homogenized mixture is suspended in 20 mL of **pre-diluted** extraction buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes.
3. The samples are centrifuged for 10 minutes at 2000 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. Due to high matrix effects samples containing more than 50% pure beef should be further diluted 1 + 1 with **pre-diluted extraction and sample dilution buffer.**
5. **100 µL** of particle-free solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the **pre-diluted extraction and sample dilution buffer** is necessary. The additional dilution has to be considered when calculating the

concentration.

**The following sample preparation should be applied for liquid samples:**

**1 mL** of liquid sample is diluted in **19 mL** of pre-diluted **Extraction & Sample Dilution Buffer.** Afterwards the suspension is incubated for 15 min in a preheated water bath at **60°C**. To ensure good homogeneity, the samples should be shaken every two minutes. The process is continued at point 3 of solid sample extraction process.

Note:

1. Do not shake the final extract to prevent from re-suspension.
2. If after centrifugation a third layer at the top appears due to a high fatty matrix, only the middle aqueous phase should be applied to the wells.

## REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- **1X Extraction and Sample diluent buffer:** Dilute 10X Extraction and Sample diluent buffer into distilled water to yield 1X.

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards** and **samples** in duplicate into wells.
3. Incubate for **20 minutes** at **RT**.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µL** of **HRP-Antibody Conjugate** into each well. Incubate for **20 minutes** at **RT**.
6. Aspirate and wash wells as step 4.
7. Add **100 µl** of **TMB mixture** to each well. Incubate for **20 minutes** at **room temperature** in dark.
8. Add **100 µl** of **Stop Solution** to each well.
9. Read the OD with a microplate reader at 450 nm **immediately**.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

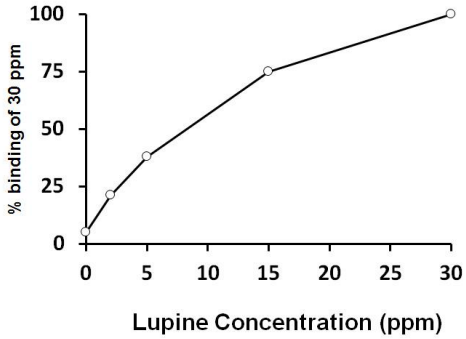
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.




## QUALITY ASSURANCE

### Sensitivity

The limit of detection (LOD) of the Lupine test is 0.2 ppm for the standard curve.

Validation experiments with common matrices resulted in the following LODs [ppm].

| | |
|---|---|
| Sausage | 0.2 |
| Bread | 0.3 |
| Orange juice | 0.7 |
| Ketchup | 0.1 |
| Croquette | 0.2 |

The limit of quantification (LOQ) of the Lupine test is 2 ppm.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

## Specificity

For the following foods no cross-reactivity could be detected:

| | | |
|---|---|---|
| Almond | Apricot | Barley |
| Bean, white | Beef, cooked | Beef, raw |
| Bovine gelatin | Brazil nut | Buckwheat |
| Cabbage, white | Caraway | Cardamom |
| Carob gum | Carrot | Cashew |
| Cayenne | Celery | Chestnut |
| Chia | Chicken | Chili |
| Cherry | Cod | Coconut |
| Corn | Cow's milk | Cumin |
| Dill | Duck | Egg |
| Fennel | Flaxseed | Garlic, fresh |
| Garlic, granulated | Ginger, fresh | Ginger, ground |
| Gliadin | Goat's milk | Garden cress |
| Guar gum | Gum arabic | Hazelnut |
| Horseradish | Kiwi | Lamb |
| Leek | Macadamia | Mustard |
| Oats | Onion | Orange |
| Paprika | Peach | Pea |
| Peanut | Pepper, black | Pine seed |
| Pistachio | Poppy | Pork |
| Potato | Prawn, cooked | Prawn, raw |
| Pumpkin seed | Radish | Rapeseed |
| Rice | Rye | Saccharose |
| Sesame | Shrimps | Split pea |
| Strawberry | Sunflower seed | Thyme |
| Tofu | Tomato | Turkey |
| Turmeric | Walnut | Wheat |

For the following commodities of the table above the results were between 0.5*LOQ and LOQ of the kit. So, it cannot be completely excluded that these matrices may provide values above the LOQ in specific cases:

| | | |
|---|---|---|
| Bean, white | Ginger, ground | Split pea |
| Cayenne | Guar gum | Thyme |
| Cherry | Hazelnut | |

The following cross reactions were determined:

| | |
|---|---|
| Adzuki bean | 0.0003% |
| Chickpea | 0.0003% |
| Cinamon | 0.0005% |
| Clove | 0.0009% |
| Cocoa | 0.0003% |
| Fenugreek | 0.0006% |
| Kidney bean | 0.0003% |
| Lentil | 0.0003% |
| Nutmeg | 0.0003% |
| Pecan | 0.0003% |
| Plum | 0.0003% |
| Soy flour | 0.0065% |
| Soy lecithin | 0.0003% |

### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6-9% and the CV value of inter-assay precision was 5-9%

### Recovery

| | |
|---|---|
| Sausage | 99% |
| Bread | 113% |
| Orange juice | 104% |
| Ketchup | 98% |
| Croquette | 111% |